ORIGINAL RESEARCH

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Improved weight gain in pigs using levamisole as an immunomodulator

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Summary

Objective: To determine the effect of two different treatment regimens of levamisole on average daily gain (ADG) from birth to weaning and to 7 weeks of age and from mortality and culling up to the age of 7 weeks, and its effect on the proportion of circulating lymphocytes.

Methods: Piglets (N = 1606) from a commercial segregated early-weaned (SEW) herd were randomly assigned either to receive one injection of levamisole within 24 hours of birth (T1 group), to receive levamisole once within 24 hours of birth and again at weaning (T2 group), or to a control group. Pigs were individually weighed within 24 hours of birth, at weaning, and at 7 weeks of age. Differential leukocyte count (DLC) was measured in the peripheral blood of 121 pigs before and after each treatment to examine the effect of levamisole on the proportional lymphocyte count.

Results: The treated pigs weighed significantly more than the control pigs at weaning ($P < .001$). From birth to 7 weeks of age, both the T1 and the T2 group gained significantly more ($P < .001$) than the control group. Neither mortality nor culling differed significantly among treatment and control groups. Treated pigs had a higher proportion of lymphocytes 24 hours after the first treatment than the control pigs ($P < .05$). There was no difference in the proportion of lymphocytes 24 hours after the second treatment.

Implications: Treatment with levamisole at birth and at weaning is associated with an increase in average daily gain (ADG).

Keywords: weight gain, weaning, levamisole, immunomodulation, pig, SEW, ADG

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Piglets are capable of responding to antigenic stimulants even during their intrauterine life, but they have an immature immune response at birth. Newborn pigs are usually born without circulating immunoglobulins,[1] and immunoglobulin formation is still poorly developed or immature for several weeks.[1] Piglets receive immunity from the dam by passive transfer of colostral antibodies, but colostrally derived serum antibodies decrease from 1–2 days of age onward.[1] The intestinal tract of the newborn piglet is capable of absorbing colostral immunoglobulin molecules during the first 36–48 hours postpartum.[2]

Birth and weaning are two major stresses in the life of the pig. At these critical events, piglets have little resistance to microbial infections. Early-weaned pigs may also be particularly susceptible to disease during the early postweaning phase. Stresses, such as transport to the nursery, can enhance the possibilities of infection and may immunocompromise pigs (Bourne. *Proc of the Nut Soc*. 1973;32(3):205–215).[3]

Levamisole has been used as an immunomodulator in humans and animals[4] since its immunomodulatory effect was first demonstrated in 1971.[5] On the basis of its chemical properties and immunomodulation ability, this drug has been classified as a thymomimetic drug.[6] It has beneficial effects on host defense mechanisms[7] and restores depressed immune responses in animals and humans.[8]

Levamisole derives its anti-anergic activity from its ability to improve and to restore the defective activity of cells that participate in cell-med iated immunity.[9] An individual animal's response is dependent on the dose, the time of administration, and the animal's immunocompetence. Pecoraro, et al.,[9] found that the immunostimulatory activity of levamisole is due to its ability to effect the maturation of immature leukocytes and also due to the stimulation of T-cell differentiation. One of the main cells involved in this process is the lymphocyte.[10] Levamisole has no effect on the concentration of circulating immunoglobulins or on antibody formation.[9]

Babiuk and Mishra[11] observed enhanced antibody response in calves after treating them with levamisole before transportation. This shows that levamisole can be used to prevent disease during stress. Considering the stress factors associated with the weaning, levamisole may have the same effect on piglets as in calves.

According to Bourne, et al.,[1] peak serum concentrations of IgA in piglets occur 10 hours after feeding. On this basis, it can be presumed that piglets that get less colostrum will have lower immunoglobulin concentrations and may benefit from the immunomodulatory activity of levamisole. Levamisole can also be used to enhance resistance to infection in immunologically immature newborns and in other situations in which immune system function is decreased.[10] If the immune system of early-weaned pigs can be stimulated to fight infectious

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organisms, we may be able to improve ADG in these pigs.

The objectives of this field trial were to determine the effect of two different treatment regimens of levamisole on:

- mortality and culling up to 7 weeks of age, after controlling for birthweight;
- average daily gain (ADG) from birth to weaning and to 7 weeks of age; and
- the proportional lymphocyte count.

Material and methods

Study design

We performed this field trial in a 500-sow segregated early weaning (SEW) commercial (Landrace × Yorkshire) herd. A total of 1606 piglets were individually weighed (Figure 1), identified with numbered eartags, and randomly assigned to one of the three groups:

- the control group, which received no treatment (n = 536),
- the one-treatment (T1) group, which received a single subcutaneous (SC) injection of levamisole (2.5 mg per kg bodyweight) at birth (n = 536), and
- the two-treatment group (T2), which received one injection of levamisole at birth and a second injection at weaning (n = 534).

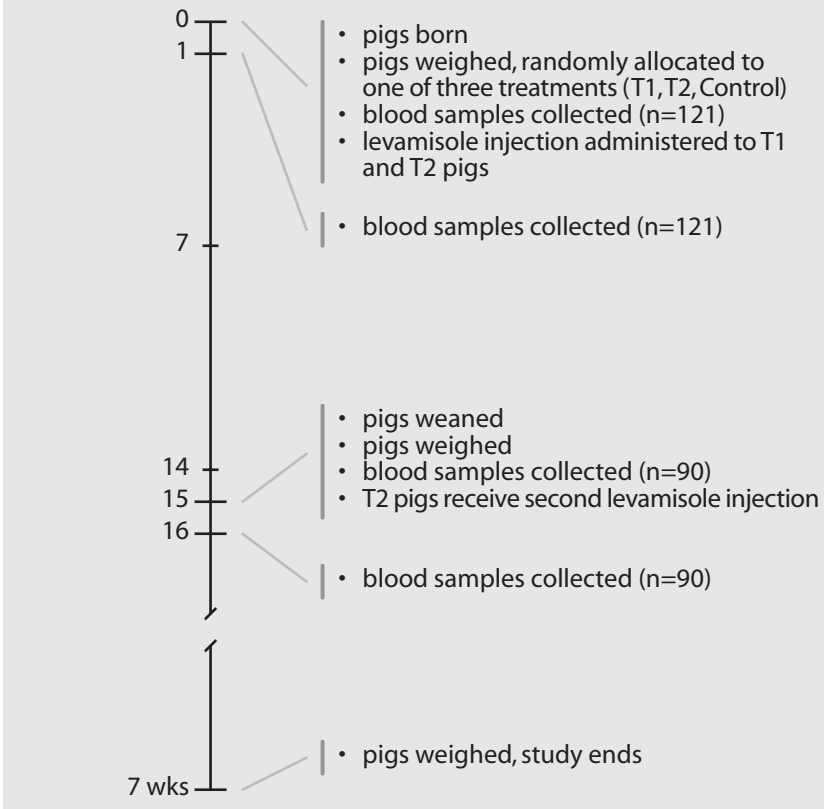
Levamisole phosphate (Tramisol Injectable™, Cynamid Canada Inc.; Markham, Ontario) was constituted in sterile water containing 9 mg benzyl alcohol per mL. An aliquot (1.65 mL) of levamisole was added to 28.35 mL of sterile water for injection, to make a concentration of 7.5 mg of active levamisole HCl per mL.

Piglets were weaned three times a week, at an average age of 15 days. The piglets were weighed at weaning and moved to a single-site nursery where one room was filled per week. All piglets stayed in the same nursery and had ad libitum access to feed and water. Piglets from each treatment group were distributed in each pen, with 25–30 pigs per pen. Piglets were not sorted according to body size, but male and female piglets were kept in separate pens. The room temperature was maintained between 38–40°C (100–104°F).

Mortality and culling were recorded by the producer, and were checked by researchers every week. The surviving pigs were weighed again at 7 weeks of age. Three hundred and thirty four pigs were weighed on a spring scale, and 957 pigs were weighed on a load cell electronic scale.

Piglets in all three treatment groups had access to the same phase-feeding ration.

Figure 1



Study timeline

Serology

Venous blood samples from 121 pigs (40 pigs per treatment group plus 1) were collected at birth and 24 hours after treatment. Blood samples were again collected from 90 pigs that were part of the original 121 (30 from each treatment group) at weaning but before giving the second dose of levamisole to the T2 group, and again 24 hours after weaning. Differential leukocyte count (DLC) was performed using a blood smear stained by the Wright-Giemsa staining process.

Statistical analysis

The unit of analysis was the individual piglet. To analyze factors associated with average gain from birth to weaning and from birth to 7 weeks of age, we used piglet weight at 15 days and 7 weeks of age as outcome variables using PC-SAS. (SAS. *User's Guide*: Statistics. Cary, North Carolina: SAS Inst., Inc., 1988) Mixed modeling was used with litter included as a random variable. The final weight variable was regressed individually on birthweight, T1 group, T2 group, and gender, retaining variables with a *P* value of .05 for the multivariate model in a backward elimination process. Because two different scales were used for weighing the 7-week-old pigs, scale was included in this model. Treatment groups were included in the model using two dummy variables, with the control group as the reference. To control for the effect of birthweight on weight gain, birthweight was included in the final regression analysis.

The general linear models (Cabaj, et al. *Vet Res Commun*.

1995;19(1):17-26) procedure was used to test the significance of differences in lymphocyte concentration among the three treatment groups. The difference in the lymphocyte concentration from birth–24 hours after treatment and from weaning–24 hours after the second treatment was used as a dependent variable to test the effect of levamisole on the lymphocyte concentration. Significant differences among the control group and two treatment groups were determined by Duncan's test (SAS. *User's Guide*: Statistics. Cary, North Carolina: SAS Inst., Inc., 1988) with a 95% confidence interval.

Results

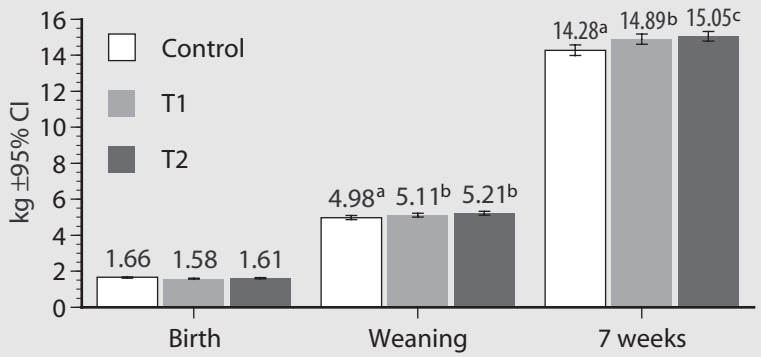
During the trial 88 pigs died (57 treatment, 31 control) and 227 were culled (147 treatment, 80 control); thus, a total of 1291 piglets were included in the final analyses.

Neither mortality nor cull rates differed among the treatment and the control groups.

The mean birthweight did not differ significantly among the three treatment groups ($P > .05$). Pigs in both the T1 and T2 groups gained significantly more from birth to weaning than control group pigs ($P < .001$) (Figure 2) even though control pigs were 63 g (2.2 oz) heavier at birth. After controlling for birthweight, treated pigs gained 292 g (0.64 lb) more from birth to weaning than control group pigs (Table 1). Piglets in the T1 group gained 668 g (1.47 lb) more than control pigs from birth to 7 weeks of age ($P < .0001$), and those in the T2 group gained 795 g (1.75 lb) more than control pigs ($P < .0001$) (Table 2). From birth to weaning, weight gain did not differ between pigs in the T1 and the T2 groups. The parameter estimate for litter in both models was 0.001. Birthweight and scale were associated with final weight ($P < .05$), whereas gender was not associated with weight gain ($P > .05$) (Table 2).

Levamisole altered the mean proportion of lymphocytes in 1-day-old pigs but, not in weaned pigs (Figure 3). There was a 12.53% increase in the proportion of lymphocytes 24 hours after treatment at birth.

Figure 2



Average weight gain

abc Within each age, values with different superscripts differ significantly ($P<.05$).

Table 1

Variables associated with final weight at weaning

Variable	Parameter estimate	Standard error	*P* value
Intercept	1.66	0.12	.0001
Birthweight	0.98	0.07	.0001
Treatment	0.29	0.05	.0001

Adjusted $R^2 = 0.37$

Table 2

Variables associated with final weight at 7 weeks of age

Variables	Parameter estimate	Standard error	*P* value
Intercept	9.27	0.40	.0001
Birthweight	2.20	0.21	.0001
One treatment	0.85	0.19	.0001
Two treatments	0.91	0.19	.0001
Scale	1.39	0.26	.0125

Adjusted $R^2 = 0.17$

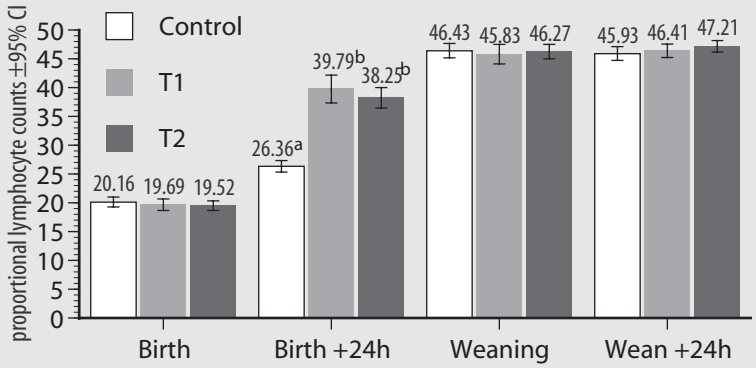
Discussion

Although the mortality on the study farm was comparatively low, there was a higher-than-normal percentage of culls before weaning. The small litter sizes and low number of pigs in the lower birthweight range may have contributed to the lower mortality at this farm. Sharpe[12] has observed that the most predominant causes of postnatal death in piglets are crushing by the sow and starvation, and that low birthweight is also an important factor contributing to mortality before weaning.[12] The lack of an observable difference in mortality among the control, T1, and T2 groups in our study contrasts with the observations of Hennessey, et al., who observed a lower mortality rate in levamisole-treated pigs in a controlled trial.[13] Because our trial was performed in a commercial herd, we were not able to control for other factors—such as physical injury, crushing by sow, and hypoglycemia—that are associated with piglet mortality. The owner did not record the cause of mortality.

Culling a piglet was the owner's decision according to the commercial value of piglets. The reasons pigs were culled included lameness, physical injury, and hypoglycemia. Pigs that were lost to follow-up after weaning were also included in the culled pigs. Birthweight plus levamisole explains 37% of the variation in weaning weights.

Pigs weaned at an early age have fewer delayed-type hypersensitivity and lymphocyte proliferative responses than pigs weaned at an older age.[14] Michaut and Dechambre[15] found a decreased immune responsiveness in early-weaned mice. The reason for decreased cellular immune response in early-weaned pigs is not clear.[13] There may be many factors, including nutrition, that effect the physiological status of the piglets, compromising their immune responsiveness.[13] The decreased cellular immune responsiveness in the early-weaned piglets may be due to defective lymphocyte regulation, caused by multiple factors.[13]

Figure 3



Proportional lymphocyte counts

ab Within each age, values with different superscripts differ significantly ($P<.05$).

A strong immunity results in better absorption and utilization of nutrients and energy from feed. It is possible that the lower degree of immunity in control pigs could result in reduced nutrient absorption and utilization, slower growth, and poorer feed conversion, which leads to a decreased ADG from birth to 7 weeks of age. The treated pigs were heavier at weaning, and seemed to maintain that extra weight gain after weaning.

The average proportion of lymphocytes in the control and treatment group was 20%, which is within the normal range at birth.[16] The proportional increase in lymphocytes we observed in treated pigs 24 hours after birth may be because levamisole enhances activation of lymphocytes by lymphokines (Il-2, interferon Gamma) or lymphokine production (Bourne. *Proc of the Nut Soc*. 1973;32(3):205-215). Levamisole has a cholinergic effect on leukocytes. This cholinergic effect causes a two- to fourfold increase of cyclic 3', 5' -guanosine monophosphate (cGMP) levels.[9] Levamisole also increases the protein and nucleic acid synthesis in resting lymphocytes (Bourne. *Proc of the Nut Soc*. 1973;32(3):205-215), which may have led to the increase in the proportion of lymphocytes we observed. The mean proportion of lymphocytes at weaning was 46% in our study, which conforms with an expected average of 50% at 2 weeks of age.[16]

Weaning piglets at less than 3 weeks of age may be associated with reduced growth rate as a result of reduced immune protection.[17] However, other factors such as nutrition, management, and environment also contribute to weight gain after birth.[18] Linear regression models were used to identify the factors statistically associated with the variation in the outcome model. In this study, multiple linear regression was used to determine whether birthweight and levamisole treatment were associated with the pig's weight at 15 days and 7 weeks of age. The R^2 value is also called the coefficient of determination. It measures the amount of variation in the outcome variable that can be explained by the variables in this model. In our study, birthweight plus levamisole treatment explained 37% (R^2=0.37) of the variation in weaning weight. The rest of the variation (63%) is explained by factors that we did not include in our model. In the model of 7-week weights, birthweight, levamisole, and scale accounted for 17% of the variation in 7-week weights. We assume that there are other important factors that explain the variability in 7-week weights. In this study, 10%–14% of the variation in weight gain could be attributed to the levamisole treatment. The low model adjusted R^2 we observed is due to other variables associated with weight gain that were not included in our model. The concentration of maternally derived serum immunoglobulin declines by 3 weeks of age,[19] and at weaning there is also a sudden withdrawal of intestinal IgA derived from maternal milk. Thus, weaned piglets need a strong immune system to protect themselves from environmental microorganisms. Chilling, lack of IgA, transportation stress, and change in diet cause a systemic immunosuppression and result in postweaning diarrhea[14] and other diseases. Wauwe, et al.,[9] have noted that the reduced immune status of early-weaned piglets seems to be an important prerequisite for levamisole's effectiveness. Levamisole may stimulate the immune system of early-weaned piglets to protect them from environmental stress and microorganisms. As a result of improved disease resistance, the nutrients absorbed from the intestine can be used for growth and weight gain.

We selected a dose of 2.5 mg per kg bodyweight, as suggested by Amery.[20] In a study of the relationships between dose and duration, he found the effect of levamisole to be optimal at a dose rate of 2.5–5.0 mg per kg. If the dose is pushed beyond 10 mg per kg, the immunomodulatory effect is more limited or there may be no effect. Levamisole breaks down to three degradation products if it is stored for a period of time at a neutral and alkaline pH,[21] or at room temperature.

Implications

- Levamisole can be used to increase the weaning weight of SEW pigs.
- Levamisole increases the weight gain of SEW nursery pigs.
- Levamisole increases the proportional lymphocyte counts and therefore may enhance immunity from birth to weaning.

Acknowledgements

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